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Remarks

This timely filed Amendment is responsive to the Office Action dated March 30, 2004. Claims 28-45 and 48-70 were pending at the time of the Office Action. All claims were rejected. Claims 28, 29, 31, 66-68 and 70 have been amended herein. No new matter has been added.

Claims 68-70 (drawn to a method) were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-36 of U.S. Patent No. 6,406,745 to Talton ("'745") in view of U.S. Patent No. 5,499,599 to Lowndes et al. ("'599"). Claims 28, 30-45, 48, 50-54, and 59-61 (drawn to coated medicament) were rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,223,244 to Moro et al. ("'244"), in view of U.S. Patent No. 5,976,577 to Green et al. ("'Green"). Claims 28, 30-45, 48-61, and 66-67 were rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,972,388 to Sakon et al., ("'Sakon) in view of Green. Claims 28, 30-45, 48, 59-61, and 66-67 were rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,855,913 to Hanes et al., ("Hanes") in view of Green. Claims 62-65 were rejected under 35 U.S.C. §103(a) as being unpatentable over Moro or Sakon or Hanes in view of U.S. Patent No. 6,277,364 to Bucks et al., ("Bucks"). Claims 29 and 68-70 were rejected under U.S.C. §103(a) as being unpatentable over Moro or Sakon or Hanes in view of Lowndes and further in view of Green.

Applicants and the undersigned, attorney Neil Jetter, wish to thank Supervisory Examiner Thurman Page for participating in a teleconference on Monday May 24, 2004 with the undersigned and inventor Dr. James Talton. Applicants' claimed invention was

reviewed and compared to the cited art, comprising Moro, Hanes, Sakon and newly cited Green. Applicants described their important pioneering technology comprising use pulsed laser ablation to coat core drug particles and how earlier conventional coating technologies, such as spray on processes, could not provide the claimed continuous nanoscale coatings on small (< 50 μm) core drug particles.

The Examiner requested clarification regarding claim 45. Claim 45 was intended to be cancelled in the previous Reply filed April 7, 2003 which requested cancellation of claim 45-47. Claim 45 had recited "The medicament according to claim 28, wherein said coating layer is a continuous layer". This subject matter is subsumed by current claim 28 and was intended to be, and should be, cancelled.

Before reviewing the cited art, Applicants will first review the claimed invention as now recited in amended claim 28. Amended claim 28 recites a medicament comprising a plurality of coated drug particles, each coated drug particle having an average particle size of less than 50 μm in diameter, the surface of the particles comprising at least a first coating layer. Support for various coated drug particles thicknesses including 50 μm can be found on page 11, line 26 to page 12, line 10. The coating layer is a continuous and non-porous layer. The coating particles are biodegradable or biocompatible. The biodegradable or biocompatible coating layer provides controlled drug delivery. The average thickness of the coating layer is from 1 to 500 nm. Amended claim 68 recites a related claim relating to a method of preparing a coated drug particle using a process comprising pulsed laser ablation.

Although Applicants disclose that the coating can be either continuous or discontinuous as correctly noted by the Examiner, Applicants' claimed continuous and

non-porous coating layer is generally a preferred embodiment as it maximizes sustained release of the drug. Although Applicants agree with the Examiner that there is motivation to achieve a continuous coating layer on drug particles "to obtain a sustained or controlled rate of delivery of the active ingredient", Applicants achieve this desired claimed continuously coated drug particle using a process comprising *laser ablation* which permits formation of continuous nanoscale coatings on 50 μm (or less) core particles. Such coated particles are not disclosed or otherwise obtainable by any methods disclosed in any of the references cited herein, such as Moro, Sakon and Hanes (spray-drying) or Green (freeze-drying), whether alone or in combination.

The claimed coated particle size advantageously includes the particle size range suitable for inhalation, which is generally accepted to be less than about 20 to 50 μm. For example, amended claim 31 recites the average coated particle size is less than 20 μm in diameter, claim 32 recites the average coated particle size is less than 10 μm in diameter, while claim 33 recites the average coated particle size is less than 1 μm in diameter.

Such thinly coated medicaments are not possible using other known methods for coating core drug particles (see Applicants' background; page 1, lines 23-30 and Green as noted below). Core drug particles having very thin (including nanoscale) continuous and non-porous coatings are evidenced by sustained release profiles which are provided throughout Applicants' specification as well as by Example 1, page 33, lines 18-20. Porous coatings, such as inherently resulting from *solvent evaporation in spray processing when used to form nanoscale coatings* as claimed by Applicants, clearly cannot provide sustained release profiles. Applicants have attached a paper (Maa et al, 1996, Intl. J. Pharmaceutics 144:47-59) and U.S. Pat. No. 5,437,889 to Jones that clearly

demonstrate that when spray processing is used, because the typical droplet size generated is 10-20 microns, the core particles must be at least about 75 to 100 μm or larger to obtain continuous coatings.

For example, Col 7, line 60 of Maa et al. clearly discloses this limitation of spray coating to core particles > about 100 microns:

> The spray-coated powders can be formed using any standard spray-coating processing apparatus. In this regard, batch-type fluid-bed processors have long been used to perform drying, granulation, and coating operations in the pharmaceutical industry for preparing solid dosage forms. Olsen, K. W. (1989) "Batch fluid-bed processing equipment: A design overview," Part I., Pharm. Technol. 13:34-46, Olsen, K. W. (1989) "Batch fluid-bed processing equipment: A design overview," Part II., Pharm. Technol. 13:39-50. With the advent of the Wurster spray coater, seed particles as small as 50 μm in size can, at least in theory, be coated. Iyer et al. (1993) Drug Devel. Ind. Pharm. 19:981-989. *However, to date, the spray coating of seed particles having an average size of 100 μm or less has been limited, particularly for protein or peptide pharmaceuticals.* (italics for emphasis only)

U. S. Pat. No. 5,437,889 to Jones is entitled "Fluidized bed with spray nozzle shielding". Column 7, line 55 of Jones discloses the minimum core particle size limitation of spray coatings:

> The surprising result of these experiments was the ability to coat the small particle size material without granulating the substrate. Comparative SEM's at the same magnification show the small fraction of the particle size distribution being incorporated into the coating on the large fraction of the distribution but then the material ceases to agglomerate and at 10% coating the particle size is very similar to the 4% sample. The average size of the end coated particles being significantly less than 100 microns, the current accepted lower limit for discrete fine particle coating. It was postulated from this observation that the minimum particle size for discrete coating might be effected by the substrate/spray pattern contact and could be significantly reduced by limiting that contact while the spray pattern is still developing.

Returning to Applicants' invention, Applicants' Example 2 (beginning on page 34, line 28) demonstrates an improved sustained release rate profile compared to the uncoated drug particles. A 90% release occurred at approximately 12 hours (for coating at 2 hertz) and beyond 24 hours (coating at 5 hertz), compared to uncoated drug particles

that reached 90 % release at approximately 2 hours (see Figs. 6 and 7). Similarly, in Example 3 (page 36, lines 11-14), in vitro dissolution of coated rifampicin reached 90% release after 6 hours compared to 90% release after 15 minutes for the uncoated rifampicin (Fig. 8).

The laser ablation process disclosed in Applicants' application can be used to produce very thin (1 to 500 nm) continuous and non-porous coating layers which are exclusive of the drug provided by the drug particles based on the laser ablation method (where the core drug particles are not in a solution or exposed to laser radiation), on core drug particles less than 50 μm in diameter. Amended claim 29 now recites this aspect of the invention with the recital "wherein said coating layer is exclusive of said drug provided by said drug particles". Solution based methods, invariably include some drug in the coating due to varying degrees of solubility of the drug in the solution.

Turning now to rejections based on cited art based on cited art, former claim 28 was rejected based on Moro, Sakon or Haynes in view of newly cited Green. According to the Examiner Moro, Sakon, and Haynes "do not explicitly teach that the coating layer is a continuous and non-porous layer". Regarding Moro, Sakon, and Haynes the Examiner asserts:

> It is the Examiner's position that there is no criticality observed in the use of Applicant's continuous or non-porous layer since the instant specification permits use of both a continuous and discontinuous coating layer (see specification pgs. 2 and 5, lines 29-32). Furthermore, it is deemed obvious to one of ordinary skill in the art to employ a continuous and non-porous coating layer to obtain a sustained or controlled rate of delivery of the active ingredient. Such skill is evident from the reference of Green et al. (see below).

Thus, Green is used by the Examiner in an attempt to make up for the deficiencies of Moro, Sakon, and Haynes.

According to the Examiner regarding Green:

Green et al. teach a process for preparing rapidly disintegrating solid dosage forms containing drug particles, wherein the drug particles may be coated or uncoated with a water-insoluble polymer or lipid material, resulting in a dosage form that exhibits delayed release of the drug for a sufficient period of time to provide for controlled or sustained release of the drug after swallowing. The drug particles have a size such that the coatings can be formed thereon which are sufficiently intact and continuous to prevent or minimize loss of drug during processing. The coarse drug particles have an average particle size up to about 500 μm. In this size range, it is possible to apply a uniform intact coating on the particle in order to achieve efficient freeze-dried dosage forms with slow drug release rate (see reference cols. 1, line 5-col. 3, line 22); (col. 5, lines 1-47).

The Examiner then concludes regarding Moro in view of Green:

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the combined teachings of Moro et al. and Green et al. because Moro et al. teach an active ingredient formulation (i.e. glycyrrhizinate) whereby a core powder is coated and covered by a sheath powder and similarly Green et al. teach a dosage form comprising drug particles that are either coated or uncoated and if coated, provide a continuous intact coating on the drug particles in order to provide a sustained release of the active ingredient. The expected result would be a continuously coated, non-porous pharmaceutical formulation that exhibits a sustained release of the active drug material, as similarly described by Applicant(s).

Analogous conclusions of obviousness are made by the Examiner with respect to Sakon in view of Green, and Hanes in view of Green. Applicants respectfully disagree with the Examiner's obviousness assertions regarding Moro, Sakon, and Haynes when combined with Green for reasons described in detail below.

First, Applicants' provide a useful table regarding the cited references:

	Green	Moro	Sakon	Hanes	Invention
Core Particle size	50-400 microns	0.1-100 microns	0.5-10 microns	5-30 microns	<50 microns
Includes drug as core particle?	yes	no	yes	yes	yes
Coated Core particle?	yes	yes	yes	yes	yes
Nanoscale coating?	no	yes	no	no	yes

Applicants will not review Moro, Sakon and Hanes in detail because such a review was provided in Applicants' reply filed April 7, 2003. However, salient points regarding these references are repeated below for convenient reference.

Spray drying is a process by which a plurality of species are generally first dissolved in a solvent containing solution. In some processes, one or more species may be insoluble in the solvent containing solution.

Moro discloses a dry mixing process to form inert particles and potentially a drug in solution which are then spray dried onto a skin surface. The solution is sprayed onto a skin surface and then drying takes place. Although in Example 10 Moro discloses glycyrrhizinate which can be used as an anti-inflammatory, the composite powder is granular tetrafluoroethylene (1 μm) with a 0.1 μm kaolin coating, neither of which are drugs. The composite powder is insoluble in the disclosed mixture. The only drug (potassium glycyrrhizinate) is freely soluble in the disclosed solvents of ethyl alcohol and water. Upon spraying and drying, a random mixture of potassium glycyrrhizinate, tetrafluoroethylene and kaolin results.

No drug particles are ever coated by Moro as the core particles are inert particles, such as silica particles. Notwithstanding the lack of continuous coatings provided by Moro's process, the resulting particles produced by Moro's process being drug in one embodiment on inert core are the *inverse* of Applicants' claimed particles (biodegradable coating layer on core drug particle).

In Sakon and Hanes, spray-drying occurs by spraying into an open chamber where the particles dry rapidly from a heated air-flow, generally from below, and the particles are suspended to aid drying. *Either way, the solvent and other volatile species*

rapidly evaporate to leave a random mixture of the remaining non-volatile species intermixed, with the resulting layer formed having significant porosity from the solvent evaporation process. No distinguishable coating layer is formed on any species since the arrangement of particles following drying is random, *being a porous continuous phase (e.g. polymer) having a plurality of core particles therein.* Accordingly, as known by those having ordinary skill in the art, spray drying does not form coated particles, and clearly cannot provide Applicants' claimed medicament comprising < 50 micron core drug particles coated with a nanoscale continuous and non-porous coating layer as evidenced by the dissolution profiles provided in Applicants' specification.

Dry mixing is another process that is quite distinct as compared to laser ablation, and produces a mixture of particles which are also quite distinct as compared to the claimed coated drug particles. In dry mixing, as the name suggests, two (or more) distinct powders are poured into a bowl and stirred or placed in a bead mill to randomize the mixture so that sampling any discrete volume within the overall sample has the same percentage of the respective components. As known by those having ordinary skill in the art, the resulting particle mixture in dry mixing is always porous and agglomeration of like particles is also generally present.

Green discloses a process for preparing an oral solid rapidly disintegrating freeze-dried bulk dosage form (tablet) of a pharmaceutically active substance having an unacceptable taste. Prior to freeze drying, a suspension of uncoated or coated coarse particles of a pharmaceutically active substance is disposed in a carrier material and is then cooled to reduce the viscosity and minimize release of the active substance during processing, as well as beyond the point of disintegration of the form in the mouth, to

minimize bad taste from the drug. The continuous phase (e.g. water) is removed and the resulting final composition is generally 1 mm or greater in size discrete units containing up to 250 mg of the drug (col. 6 line 49), such as tablet shaped articles with small (uncoated or coated) drug particles dispersed throughout, analogous to a "plum pudding" of drug particles in a sea of freeze dried coating material (such as gelatin). Example 2 of Green is copied below in its entirety:

Example 2

Material	%
Purified water	74.99
Gelatin	3.00
Mannitol	2.50
Coated paracetamol	19.51
FDC Blue No. 2	0.0025

The gelatin and mannitol were added to the water and heated to 40 C. to dissolve before allowing to cool to 23 C. The mix was gradually added to the coated paracetamol (200 μm particles, 82% potency, coated with a water insoluble polymer) with manual mixing until a fluid suspension was formed. The process was the same as in Example 1. Viscosity values obtained are quoted at a shear rate of 500 s^{-1}. 0.5 ml aliquots (80 mg paracetamol) of the suspension were also dosed manually using a Gilson pipetteman into preformed PVC/PVdC blisters which were then frozen rapidly at -80 C. Freeze drying was then performed using a standard cycle. The blisters were then sealed with foil.

The temperature of the mix was then adjusted, and after allowing to equilibrate for 45 minutes the measurements and dosing was repeated.

Temperature (.degree. C.)	Viscosity (mPa s)	% sedimentation in 5 mines	Tensile Strength Nmm^{-2}	Disintegration time(s)
23.0	36.04	33	0.536	1.4
21.0	42.66	8.9	0.561	1.4
18.7	53.08	0	0.553	3.4
17.6	51.01	0	0.598	3.7
14.9	73.06	0	0.630	3.5

The results demonstrate the increase in viscosity of the suspension as the temperature is decreased. The disintegration times of the units do increase very slightly but are still rapid at viscosity levels sufficient to prevent any sedimentation in 5 minutes. When tasted, the units dispersed in the mouth with no bitter taste.

Thus, the particle coating step, and the resulting coated drug particles, which is the focus of Applicants' claimed invention, is outside the scope of Green. Green clearly does not form discrete coated drug particles as claimed by Applicants. Green only uses coated (or uncoated) drug particles in his suspension process to form a bulk dosage form (fast-dissolving tablet) incorporating a plurality of drug particles.

Green relies on "current coating techniques", such as to provide the paracetamol (200 μm particles, 82% potency) coated with a water insoluble polymer disclosed in Example 2 above, and provides the following teaching regarding the same (col. 3, lines 9-21):

> Current coating techniques are able to effectively coat particles greater than 100 μm, whereas particles less than *100 μm may not have an intact coat*, which will result in rapid release of the drug once in suspension. Coating of larger particles therefore decreases the rate of release of drug. Typically, according to the present invention, the coarse particles may have a size of up to 1 millimeter, although the average size is generally up to about 500 μm, for example 75 to 400 μm, more usually in the region of about 100-300 μm. *In this size range, it is possible to apply a uniform intact coating on the particle in order to achieve efficient freeze-dried dosage forms with slow drug release rate.* (italics for emphasis only).

In addition, col. 5, lines 27-52 of Green discloses the following:

> Generally, the coating on the particles is a polymer or lipid material and serves to prevent loss of the pharmaceutical agent during processing, as well as delaying release of the pharmaceutically active substance beyond the point of disintegration of the form in the mouth. Any suitable polymer or lipid or combination can be used as the coating material. Examples of suitable polymers include cellulose and derivatives such as ethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, methylcellulose, cellulose acetate, cellulose acetate phthalate, hydroxypropylmethylcellulosephthalate, acrylic derivatives, such as polymethacrylates, polyglycolic--polylactic acid, polyvinylalcohol, gelatin, collagen and polyethyleneglycol. Examples of suitable lipid materials include waxes such as beeswax and lanolin, stearic

acid and derivatives such as glycerol esters, fixed oils, fats, phospholipids, and glycolipids.

Such coatings are well known to persons skilled in this art. Persons skilled in the art could also readily provide coatings having a particular dissolution time so as to ensure that drug release is prevented until required. (italics for emphasis only)

Thus, Green does not cure the deficiencies of Moro, Sakon and Hanes. Significantly, as noted above, Green provides evidence that core particles obtained using known techniques (which although not explicitly disclosed would include the well known spray techniques disclosed in Moro, Sakon and Hanes references) must be at least 75 μm, more usually in the region of about 100-300 μm, to achieve a "uniform intact coating on the particle" to achieve "efficient freeze-dried dosage forms with slow drug release rate". A continuous coating is important to Green because Green needs continuous coated particles in his suspension to limit the drug entering the suspension and consequently in his resulting freeze dried coating. As amended, Applicants' claim 28 now recites the coated drug particle has a diameter of less than 50 μm, the coating layer being a continuous and non-porous layer, having an average thickness of the between 1 and 500 nm.

Although size changes alone do not generally impart patentability to a claimed invention over otherwise similar structures based on the MPEP and precedential case law, size changes can provide patentablity, such as if unexpected and/or new results are achieved by a size change, such as a change in function(s) or application(s). Assuming *arguendo* that the cited references can be used to form continuously coated core drug particles, such as micron scale coatings on 100 μm or more core drug particles, MPEP 2144.04 entitled Legal Precedent as Source of Supporting Rationale, under IV. CHANGES IN SIZE, SHAPE, OR SEQUENCE OF ADDING INGREDIENTS clearly

supports patentablity of the claimed invention. MPEP 2144.04 IV cites In *Gardner v. TEC Systems, Inc.*, 725 F.2d 1338, 220 USPQ 777 (Fed. Cir. 1984), *cert. denied*, 469 U.S. 830, 225 USPQ 232 (1984). In Gardner the Federal Circuit held that a claimed device recitation of dimensions (e.g. smaller) relative to a device having different dimensions (e.g. larger) would be non-obvious if it performed differently than the prior art device, and thus would be patentably distinct over the prior art device.

The size of Applicants' claimed nanoscale thick (1 to 500 nm) coated drug particles (< 50 μm in diameter) provide unique biological responses. While particles for inhalation are typically as large as 10 microns (Sakon '388), geometrically large, aerodynamically light particles up to 30 microns (5-30 μm in diameter) have also been described (Hanes '913). Because the nasal passages are larger, particles for nasal delivery are typically up to 40 to 60 microns, with larger particles impacting in the initial turbinate. Since an increase in particle size, such as above 50 microns, would reduce the delivery of the appropriate sized particles, spray-coating techniques cannot be used.

Particles according to the invention also provide rapid and complete dispersion, as well as sustained release, for several hours. A hypothetical 'micron-scale' continuously coated drug particle (>100 μm) is clearly too large to be used for inhalation (depositing in the mouth or throat) and release of the core drug over many hours to several days (if continuously coated using spray drying). Accordingly, amended claim 28 and its respective dependent claims are believed to be patentable over the cited art.

Amended claim 50 (pharmaceutical formulation), amended claim 62 (kit) include the recital of amended claim 28 therein. Amended claim 66 has been amended to become

consistent with claim 28. Accordingly, these claims and their respective dependent claims are believed to be patentable over the cited art.

Regarding method claims 68-70, claims 1-36 of the Talton patent (U.S. 6,406,745) were cited under non-statutory obviousness-type double patenting together with newly cited U.S. Pat. No. 5,499,599 to Lowndes et al. Amended claim 68 recites a pulsed laser ablation process to form a coated drug particle having an average particle size of less than 50 μm in diameter, using a vacuum between 1 mTorr and 1 Torr. According to the Examiner:

"the only significant distinction between instant claims 68-70 and Pat. '745 is that '745 recites a pressure of 'about 10 Torr or higher' whereas the instant amended claim 68 recites 'between 1 mTorr and about 1 Torr' ". The Examiner also asserts that Lowndes "demonstrates the co-relationship between varying pressure and varying coating thickness, it is deemed obvious to one of ordinary skill in the art to adjust the pressure or Torr in order to obtained the desired or intended coating thickness." Although Dr. Talton is an inventor on both Talton '745 and the present invention, Applicants respectfully disagree that the claimed invention is obvious based on Talton '745 as explained below.

Talton discloses methods of coating core materials by providing target materials and core materials; ablating the target materials to form ablated particulate target materials; and coating the core materials with the ablated particulate target materials. The method is performed at *a pressure of 10 Torr or higher*. Methods of coating particles with nanometer to multiple nanometer thick coatings in atmospheric pressure, and using pneumatic fluidization, are also provided. (italics for emphasis only)

Although Talton '745 discloses use of a vacuum of as low as 10 Torr, Talton teaches preferentially ablating at atmospheric pressure (760 Torr). According to col. 7, line 1 to line 12:

The invention is operated such that the coating chamber has a pressure of around atmospheric pressure, which may be a pressure as low as about 10 Torr to as high as about 2500, or any pressure in between. Preferably, the pressure in the coating chamber is greater than about 20, or 30, or 40, or 50 Torr, more preferably greater than about 100 or 500 Torr, and most preferably greater than about 700 Torr. Preferably, the pressure in the coating chamber is less than about 1000, more preferably less than about 900, and most preferably less than about 820. *In a most preferred embodiment, the pressure in the coating chamber is about 760 Torr, or atmospheric pressure.* (italics for emphasis only)

Moreover, Talton' 745 teaches significant advantages of operation at a comparatively higher pressure as compared to the claimed method. According to col. 6, line 44 to line 48:

Operating the coating process at approximate atmospheric pressure allows for a continuous production process. Rather than needing to apply a vacuum on each batch for coating, the process of the present invention, operated at near atmospheric pressure, allows for continuous processing.

Thus, Talton '745 does not disclose or suggest an ablation process under a vacuum of less than 10 Torr, and in fact teaches away from use Applicants' claimed < 1 Torr (mTorr) vacuum level range. Based on the many advantages associated with the high pressure process (including atmospheric pressure) disclosed in Talton '745, such as a high deposition rate, there would be no motivation to look to another PLD reference for alternate processing conditions.

Lowndes discloses a method for growing a deposit upon a substrate of semiconductor material which involves the utilization of pulsed laser deposition techniques within a low-pressure gas environment. The substrate and a target of a first material are positioned within a deposition chamber and a low-pressure gas atmosphere is

developed within the chamber. The substrate is then *heated*, and the target is irradiated, so that atoms of the target material are ablated from the remainder of the target, while atoms of the gas simultaneously are adsorbed on the substrate/film surface. The ablated atoms build up upon the substrate, together with the adsorbed gas atoms to form the thin-film deposit on the substrate. By controlling the pressure of the gas of the chamber atmosphere, the composition of the formed deposit can be controlled, and films of continuously variable composition or doping can be grown from a single target of fixed composition.

The substrate heating taught by Lowndes is consistent throughout the patent and the disclosed substrate temperature range is quite high relative to room temperature. For example, col. 3, line 41-46 of Lowndes discloses the following:

> The substrate 16 is heated to an elevated temperature (normally between about 250 degree C. and 450 degree C.) so that a film with desired properties can be grown. Substrate heating also may assist the gas of the vessel atmosphere to be dissociated to its atomic or molecular level (depending upon the species of gas), and in any case, the gas will be adsorbed on the substrate 16.

Thus, although Lowndes mentions use of a vacuum level as low as 1 Torr, Lowndes combines the low vacuum level with substrate heating "normally between about 250 degree C and 450 degree C" to overcome the limitations of a low deposition pressure. Applied to Applicants' coated drug particles, the substrate is a drug particle. Such high temperatures are clearly incompatible with virtually any drug. Thus, one having ordinary skill in the art at the time of the claimed invention familiar with Talton '745 would not look to Lowndes which discloses use of a pressure below 10 Torr because of the required substrate heating consistently taught by Lowndes *which is clearly incompatible with core drug particles.*

Applicants have found that although a significantly lower deposition rate generally results from use of a relatively low pressure, use of a lower pressure (less than 10% of the lowest pressure disclosed in Talton '745) surprisingly provides significantly smaller particles and a coating layer having higher purity (less ambient inclusion) as compared to the higher pressure (including atmospheric pressure) disclosed in Talton '745. Accordingly, Applicants submit that the obviousness-type double patenting rejection of claims 68-70 based on Talton '745 should be removed.

Applicants have made every effort to present claims which distinguish over the cited art, and it is believed that all pending claims are in condition for allowance. However, Applicants request the Examiner to call the undersigned (direct dial 561-671-3662) after review of this Reply if the Examiner determines that any clarification is necessary to permit issuance of a Notice of Allowance.

Respectfully submitted,

Date: MAY 28, 2004

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Docket No. 5853-186US

US005437889A

United States Patent [19]

Jones

[11] Patent Number: 5,437,889

[45] Date of Patent: Aug. 1, 1995

[54] **FLUIDIZED BED WITH SPRAY NOZZLE SHIELDING**

[75] Inventor: David M. Jones, Ramsey, N.J.

[73] Assignee: Glatt Air Techniques, Inc., Ramsey, N.J.

[21] Appl. No.: 103,129

[22] Filed: Aug. 9, 1993

Related U.S. Application Data

[62] Division of Ser. No. 783,124, Oct. 28, 1991, Pat. No. 5,236,503.

[51] Int. Cl.[6] B05D 1/24

[52] U.S. Cl. 427/185; 427/213

[58] Field of Search 427/185, 213

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Primary Examiner—Janyce Bell

Attorney, Agent, or Firm—Jacobson, Price, Holman & Stern

[57] ABSTRACT

A shield, such as an upstanding cylindrical partition, is mounted adjacent to an air source, such as an air distribution plate/screen of a Wurster system processor, whereby the open upper end of the partition is generally horizontally registered with and disposed about the upper extremity of an associated spray nozzle. The open lower end of the inner cylindrical partition is generally sealed relative to the air distribution plate/screen and operative to receive air upwardly therethrough for subsequent passing through the inner partition about the spray nozzle. The upper end of the inner tubular partition shields the initial spray pattern discharged from the spray nozzle and prevents the premature entrance of particles moving into the spray nozzle area.

9 Claims, 1 Drawing Sheet

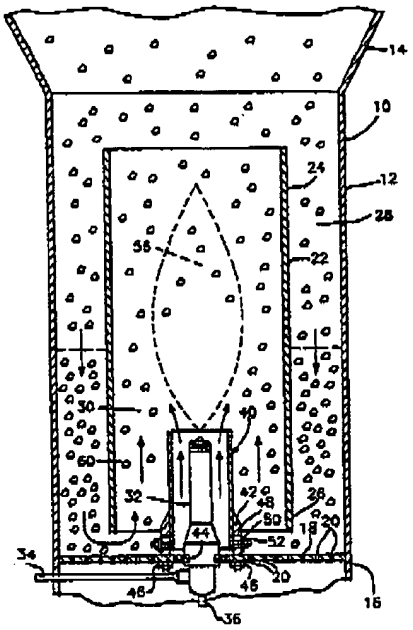

FIG. 1

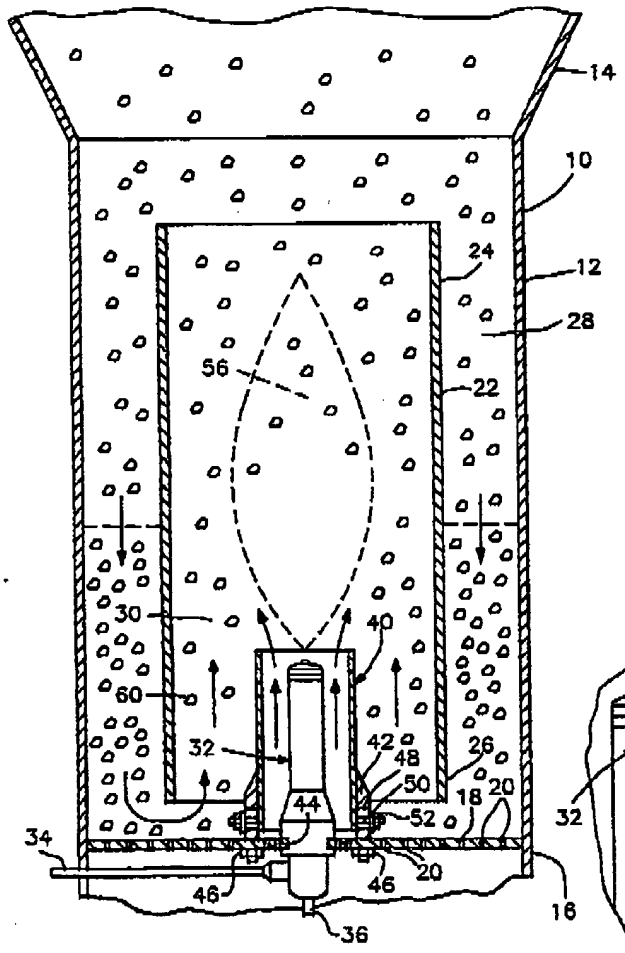
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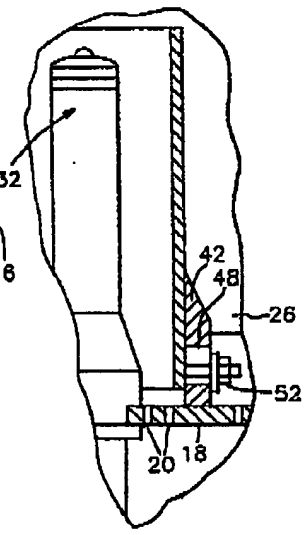
FIG. 2


32
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FLUIDIZED BED WITH SPRAY NOZZLE SHIELDING

This is a Divisional of application Ser. No. 07/783,124, filed Oct. 28, 1991 now U.S. Pat. No. 5,236,503, dated Aug. 17, 1993.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a fluidized bed system having a spray nozzle therewithin. One such system is a Wurster system type of fluidized bed processor including an outer cylindrical partition disposed within the product container and a spray nozzle within the partition. The spray nozzle itself is shielded, preferably by being surrounded by a cylindrical partition extending from the orifice plate or screen at the bottom end of the product container to shield the spray nozzle tip.

2. Description of Related Art

Various different forms of spray coaters heretofore have been provided including the Wurster system type. These previously known devices may experience turbulent air flow immediately above the spray nozzle. Particles to be coated within the system may enter the liquid discharge spray pattern before the spray pattern has fully developed. This results in uncontrolled droplet formation upon those particles that enter the spray pattern too soon and effects the effectiveness of the system resulting in excessive agglomeration and relatively extensive processing time.

U.S. Pat. No. 3,110,626 to G. L. Larsen et al. discloses an apparatus whereby coating discrete solids suspended in a moving air stream is carried out within the interior region of a velocity concentration control element mounted in the base region of a funnel-shaped coating chamber. However, such apparatus does not include any means for shielding the base of the spray pattern with an upwardly flowing column of air in order that the spray pattern may substantially develop before entrance thereinto of discrete solids to be coated.

U.S. Pat. No. 4,335,676 to Christian Debayeux et al. discloses a spouted bed granulating and/or coating apparatus wherein flow directing structure is provided to direct the gaseous flow stream in the upward direction for preventing contact and agglomeration of particles in the vicinity of the walls of the device. This patent fails to disclose structure by which the lower portion of the spray pattern is protected by an upwardly flowing column of air in order that the spray pattern may more fully develop before the entrance thereinto of particles to be coated.

U.S. Pat. No. 4,701,353 to Stanislaus M. P. Mutters et al. discloses an apparatus whereby the liquid spray material is discharged out of a central channel as a vertically closed, conical film with a thrust exceeding the thrust of the gas stream for the purpose of causing the conical film to be nebulized to very fine droplets with the aid of the surrounding gas stream. The resultant spray pattern is not protected about its initial base end by an upwardly moving column of air disposed thereabout.

U.S. Pat. No. 4,960,224 to Gustav A. Magg et al. discloses an atomizing nozzle constructed in a manner to eliminate the need to provide a metering pump or flow meter for each atomizing nozzle of an associated fluidized coating bed with the control of the flow through each atomizing nozzle being accomplished by varying the internal bore size of the flow control tubes. However, this patent fails to disclose structure for shielding the resultant spray pattern from immediate entrance thereinto of particles to be coated before the spray pattern is reasonably developed.

U.S. Pat. No. 4,858,552 to Werner Glatt et al. discloses an apparatus whereby a fluidized current carries particles, while still plastic, upwardly through a channeling device for agglomerated material disposed at a distance above the perforated base causing the particles to impinge on the underside of a rotatable means providing for shaping the agglomerated material. The Glatt et al. apparatus does not disclose structure by which the particles to be coated are shielded against entry into the initially forming spray pattern.

U.S. Pat. No. 3,196,827 to D. E. Wurster et al. discloses a tubular partition defining an upbed therein into which an air and spray discharge pattern is directed and wherein a downbed of particles in near weightless suspension is disposed outwardly of the tubular partition, the spray nozzle being disposed below the bottom of the partition and above the associated air distribution plate or screen. With this device, particles being coated are also free to immediately enter the lower beginning portion of the spray pattern.

SUMMARY OF THE INVENTION

It is understood that the dynamics of the area around the nozzle and spray cloud (often described as the coating zone) determine the overall coating speed as well as the amount of agglomeration during a fluidized bed coating process. The type of nozzles commonly used are pneumatically atomized, i.e. using a high-speed jet of air in order to break a liquid jet into small droplets and to distribute the small droplets in a cone-shaped cloud or spray pattern.

It has been observed in the course of several laboratory Wurster coating trials that material, including substrate, from the fluidization or processing air stream has been drawn into the spray nozzle liquid/air jet before the spray pattern has been fully developed. In some cases, when the material being coated has abrasive properties, it was found that the material was moving with sufficient force to cause erosion of the nozzle tip.

Accordingly, the present invention introduces a shielding or barrier means around the lower portion of the nozzle, within the upbed, for shielding the nozzle and allowing up flow of air within the shielding means around the nozzle. This ensures that particles, disposed in the product container outwardly of the barrier, are prevented from entering the spray pattern before the spray pattern is sufficiently developed. This allows the droplet density to decrease before contact therewith by the particles of the fluidized bed and, accordingly, the particle surface will be more evenly wetted preventing excessive particle agglomeration. The liquid contact with the particles can be more precisely controlled and higher spray rates can be achieved with less agglomeration.

A principal object of this invention is to shield the spray discharging nozzle of a Wurster type fluidized bed processor. The shield prevents the entry of particles into the spray pattern before the spray pattern has had an opportunity to develop.

Another object of this invention is to provide an apparatus by which particles to be coated are prevented from entering the spray pattern until such time as the

droplet density of the spray pattern has been substantially reduced.

Yet another object of this invention is to provide a columnar shield of upwardly moving air about the lower portion of the spray pattern formed by a spray nozzle of a Wurster system processor and wherein the shield may be adjusted vertically according to the spray pattern being discharged and the air flow velocity of the processing air.

A further object of this invention is to provide a shield such as that set forth in the immediately preceding object and whereby the vertical positioning of the shield may be utilized to alter the associated spray pattern.

Yet another object of this invention is to provide a coating zone within the upbed of a Wurster system coater whereby the coating zone is protected by a surrounding column of upwardly moving air in order to allow the coating zone to more fully develop and the liquid droplet density thereof to be substantially reduced prior to entry of particles into the coating zone.

These, together with other objects and advantages which will become subsequently apparent, reside in the details of construction and operation as more fully hereinafter described and claimed, reference being made to the accompanying drawings forming a part hereof, wherein like numerals refer to like parts throughout.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a fragmentary schematic vertical sectional view of a Wurster-type bottom spray coater illustrating the mounting of an inner tubular partition about the spray nozzle and projecting at least slightly above the upper extremity of the spray nozzle; and

FIG. 2 is an enlarged fragmentary schematic vertical sectional view illustrating the manner in which the interior partition may be adjusted vertically relative to the air distribution plate.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now more specifically to the drawings the numeral 10 generally designates a typical Wurster system type of coater, modified in accordance with the present invention. The coater includes a product container section 12, an expansion chamber 14 into which the upper end of the product container section 12 opens, and a lower plenum 16 disposed beneath the product container, separated therefrom through the utilization of an air distribution plate or screen 18. The upper end of the expansion chamber 14 may open into a filter housing (not shown) disposed thereabove including the necessary air filter structure and air outlet. A typical Wurster coater is described in *Encyclopedia of Pharmaceutical Technology*, Vol. 1. pp. 192–195 (1988).

The air distribution plate or screen 18 defines a plurality of air passage openings 20 through which air or gas from the lower plenum 16 may pass into the product container section 12. As is conventional in Wurster-type fluidized bed systems, the holes in the plate 18 in the area outside the cylindrical partition 22 are smaller in diameter than the holes beneath the partition 22. This results in higher air volumes and velocities in the central area than in the downbed area. Although only a single plate 18 is depicted, it should be understood that the plate 18 is typically formed of two plates, an outer annular plate and an inner replaceable plate whereby the air flow ratios between the upbed and the downbed may be changed by substituting the inner plate with a plate having different air opening configurations. Further, the inner plate may itself be formed of two stacked plates having identical air opening configurations but which are rotatable with respect to each other to change the effective open area.

The product container 12 has a cylindrical partition 22 supported therein in any convenient manner having open upper and lower ends, 24 and 26, the lower end 26 being spaced above the air distribution plate or screen 18. The partition 22 divides the interior of the product container section 12 into an outer annular downbed area 28 and interior upbed 30. A spray nozzle assembly, referred to generally by the reference number 32, is mounted at or through the air distribution plate 18 and preferably projects upwardly into the interior of the cylindrical partition 22 and the upbed 30 defined therein. The spray nozzle 32 receives a supply of air under pressure through an air supply line 34 and coating liquid under pressure through a liquid supply line 36, as is known in the art.

This invention incorporates the provision of a shield, such as an inner cylindrical partition 40 disposed about the upwardly projecting spray nozzle assembly 32. The inner cylindrical partition 40 has its lower open end snugly and telescopingly received within a tubular collar 42 secured to the air distribution plate or screen 18 about the opening 44 through which the spray nozzle assembly 32 is secured. The tubular collar 42 is secured to the air distribution plate or screen 18 through the utilization of suitable fasteners 46. The collar 42 includes circumferentially spaced axial slots 48 in which mounting studs 50 projecting radially outwardly of the inner cylindrical partition 40 are slidingly received. The mounting studs 50 have threaded bolts 53 threadedly engaged therewith whereby vertically adjustment of the inner cylindrical partition 42 relative to the tubular collar 42 may be enabled.

The spray nozzle assembly 32 discharges a spray pattern 56 of air and coating liquid. Some of the air introduced into the lower plenum 16 passes upwardly through the openings 20 formed through the air distribution plate or screen 18 below the inner cylindrical partition 40. The partition 40, as well as the tubular column of upwardly rising air about the spray nozzle assembly 32, shields the lower, beginning portion, of the spray pattern 56. The particles 60, passing upwardly through the upbed 30, are not drawn into this spray pattern. The annular column of air thereby allows spray pattern 56 to substantially develop, and the liquid droplet density of the spray pattern is substantially reduced before particles 60 enter into the spray pattern 56. By adjusting the height of the inner cylindrical partition 40 relative to the tubular collar 42, the height of the upper end of the inner cylindrical partition 40 relative to the upper extremity of the spray nozzle assembly 32 may be adjusted.

Although only a single outer partition 22, inner partition 40, and spray nozzle 32 are depicted, multiple outer partitions may be employed, each having one or more spray nozzles and inner partitions associated therewith.

Various modifications to the above-described preferred embodiment may be utilized. For example, a spray nozzle may be utilized in systems other than Wurster coater type systems where shielding is desirable. Such systems may not require the spray nozzle to be upwardly disposed; the spray nozzle may be angled with respect to the major axis of the container. The

shielded spray nozzle may also be utilized without a Wurster-type cylindrical partition 22. The shielded spray nozzle may also be within an expansion chamber instead of the product container. Further, although an air distribution plate or screen is depicted, gas flow may originate into the processor through other structural arrangements. In addition, although the shielding of the spray nozzle is preferably provided by an inner cylindrical partition 40, other shielding arrangements may be utilized. For example, shielding of the spray nozzle may be accomplished by formation of an air wall or stream that surrounds the nozzle and prevents particles from prematurely entering into the spray pattern. Alternatively, a deflector or shield may be formed integral with the spray nozzle itself to prevent particles from interfering with the developing spray pattern.

Utilizing the concepts of the invention hereinabove described, the following represents test procedures followed and results obtained made in accordance with the invention:

Materials

Sugar/cornstarch beads (Nu-pariels, Crompton & Knowles, Ingredient Technology Division, Pennsauken, N.J.) in the size range of 20-25 mesh were used as a model coating substrate for most of the trials. These beads are deemed to be a good model since many active materials are converted into pellet form for coating, or are layered onto these type of beads. Theophylline (<325 mesh, Knoll Whippany, N.J.) and potassium chloride (20-60 mesh, Mallinkrodt, St. Louis, Mo.) served as models for powder substrates.

A typical aqueous HPMC based coating solution (Opadry, Colorcon West Point, Pa. 10% coating by weight in tap water) was used throughout the trials. A green colored formula (YSI-3303N) was used for most of the experiments but was shifted to a Maroon color (Y-1-3910) to check the effects of viscosity and composition differences on the coating performance.

Equipment

All of the coating trials were carried out in a GPCG60/100 fluid bed granulator/coater (Glatt Air Techniques, Ramsey, N.J.) using either the 32" or 18" Wurster process inserts. The 18" Wurster trials utilized the standard 9" diameter partition insert, which is 22" long. The 32" Wurster trials were performed with a single partition insert 12" in diameter, 27.5" long.

The partition height was adjusted so that a thick stream of substrate flowed through the coating zone. Due to the different airflow patterns in the two sizes of Wurster inserts used the partition needed to be set at a different height for each insert. A distance of 0.75 inches from the bottom plate was sufficient for the 18" Wurster. For the 32" Wurster the partition was raised to 1.25 inches above the bottom plate to maintain a high density particle stream.

A bottom screen, stainless steel dutch weave type, 100 mesh rating was in place over the air distribution plate during all the trials. Exhaust filters, "PACF" type, rated 3-10 microns were used to retain any process fines. The filter shake cycle was set to shake for 3 s every 30 seconds in the GPCG mode. This meant that each filter shook 3 s after every 63 s of process time.

Two types of nozzles were used each with a different characteristic spray pattern development. Model #940/7-1-S25 (manufactured by Gustav Schlick Co. Coburg, Germany referred to as S-25) is supplied standard for these sizes of Wurster inserts. Model #0/4-7-1S48 (referred to as S-48) has a higher air consumption rate and fully atomizes the liquid stream distributing the droplets in a cone shaped pattern (develops spray pattern) in a shorter distance. Liquid was delivered to the nozzles with a peristaltic pump. The atomization air pressure was adjusted so that the droplet size distribution would remain the same no matter which nozzle was in use. Nozzle ports with a 1.2 mm opening were used with the air cap adjusted flush with the nozzle port end.

In those trials where the fluid flow in the coating zone was altered the fluidized particles were delayed from contacting the atomized stream by a 3" diameter cylinder partition mounted on the bottom plate and extending up 0.5" above the nozzle port height. The cylinder partition was open to the fluidizing air at the bottom.

Test Procedure

The air volume settings were determined by a pre-trial fluidization test. Air volume was then held constant for each unit throughout the trials without further optimization. For the 18" Wurster the air volume was 750 cfm (±100 cfm) and 1700 cfm (±100 cfm) was used on the 32" Wurster. For experiments on both inserts the product temperature was held between 38° and 42° C. with an inlet air dewpoint of 8° C. (±1° C.).

Each run would be allowed to come to the desired test conditions, then the spray was started at a slow rate. If after 15 minutes of spray a sample taken had less than 1% retained on the sieve specified for the test then the performance was deemed acceptable and the spray rate would be raised. The amount of the spray increase was determined by estimating the point at which 1% of the sample would be retained on the test sieve.

With the sugar beads a 20 mesh sieve was the test sieve as mentioned above. After each trial the batch was put through a 20 mesh sieve in order to remove any agglomerates. The beads passing through 20 mesh were used in the subsequent trial. For the 32" trials with sugar beads an 18 mesh sieve was used in the test in order to account for the bead growth due to repeated coating. On the 18" Wurster a starting charge of 45 kg was used compared to 200 kg on the 32" Wurster.

The experiment with theophylline as a substrate used 26 kg of raw material in the 18" Wurster. The test sieve for Theophylline was 100 mesh. With potassium chloride 50 kg were charged to the product container. At the end of the potassium chloride run the process parameters were varied in order to find the maximum possible spray rate without having more than 1% on 18 mesh. Air volume was increased from the protocol value of 750 cfm to the highest value possible, 1200 cfm. Atomization air pressure was also increased from 2.5 bar to 4.0 bar.

Experimental Program

A matrix of experiments was set up to test the coating performance with nozzle type, barrier installation, and machine size as variables. On the 18" Wurster four experiments were run with the sugar beads and the green coating: nozzle S-25 with and without the barrier in place, and nozzle S-48 with and without the barrier in place. The set of experiments using the S-25 nozzle was then repeated in order to confirm the results. Powdered substrate, theophylline and potassium chloride were used with the S-48 nozzle and the barrier in place in the last two experiments on the 18" Wurster.

In the 32" Wurster two experiments using the S-48 nozzle and spraying onto sugar beads were performed—one with the barrier in place and one without. In these trials the coating solution was switched to maroon to see if the improvements in coating efficiency were still evident with a more viscous solution.

Results and Discussion

The spray rate at 1% agglomeration was estimated from the data reported during the run on the batch sheets. Data for the matrix of 18" Wurster experiments is shown in Table 1. The data for the S-48 nozzle experiments has been averaged between the two runs.

TABLE 1

18" Wurster Spray Rate @ 1% Agglomeration (ml/min)		
nozzle	barrier	no barrier
S-25	290	90
S-48	410	220

Examination of the table reveals a dramatic improvement in spray rate at an equal level of agglomeration for use of a barrier as compared with the standard case without use of a barrier. It is also of interest that the improved efficiency by changing to a nozzle that develops the spray pattern more quickly is nearly the same as placing a barrier around the standard nozzle. This observation is in agreement with the hypothesis that the agglomeration and limitation of spray rate is caused by the premature contact of substrate with the developing spray pattern.

With a similar extraction of the data the 32" Wurster showed 1% agglomeration at 550 g/min spray without the barrier and 780 g/min with the barrier in place. Only the S48 nozzle was tested this time with the maroon coating. The efficiency increase is in similar proportions to the experiment on the 18" Wurster even though the scale of the machine and the coating characteristics were changed.

Both the theophylline and potassium chloride coated with the S-48 nozzle and the barrier in place had similar behavior during the run to a conventional machine setup. The spray rate for the potassium chloride substrate was 1050 g/min at the point of first agglomeration. Agglomeration began to occur slowly at 1250 g/m. However, at this high spray rate, the machine's drying capacity was exceeded and the desired product temperature could not be maintained. As a result, excessive surface moisture probably allowed the formulation of liquid bridges between particles ultimately leading to agglomeration. Although the acceptable spray rate for theophylline was less dramatic, the 230 g/min sprayed was higher than expected and yet did not exceed an acceptable level as in the sugar bead experiments.

The surprising result of these experiments was the ability to coat the small particle size material without granulating the substrate. Comparative SEM's at the same magnification show the small fraction of the particle size distribution being incorporated into the coating on the large fraction of the distribution but then the material ceases to agglomerate and at 10% coating the particle size is very similar to the 4% sample. The average size of the end coated particles being significantly less than 100 microns, the current accepted lower limit for discrete fine particle coating. It was postulated from this observation that the minimum particle size for discrete coating might be effected by the substrate/spray pattern contact and could be significantly reduced by limiting that contact while the spray pattern is still developing.

CONCLUSIONS

Throughout the experiments described here it is clear that limiting the contact of substrate with a developing spray pattern so that the particle surface is only exposed to spray in low droplet density areas of the spray pattern reduces the over wetting of substrate surface thereby reducing the rate of product agglomeration. Introduction of a barrier to prevent solids from entering the spray pattern and changing the nozzle design so that the pattern develops in a smaller space were shown to have a roughly equivalent effect. Substantial efficiency improvements were still evident when the barrier was used with the modified nozzle. This technique of improved solid liquid contacting was observed on two different sizes of equipment with two different coating formulations on three different substrates.

In addition to the expected result of improved coating efficiency it was also observed for the theophylline, the sample with smallest particle size, that the particle size of the coated material could be kept below normally accepted minimum sizes.

What is claimed is:

1. A method for coating product in a fluidized bed having a product container section opening upwardly into an expansion chamber and downwardly into a lower plenum chamber through a generally horizontally disposed air distribution plate/screen having openings formed therethrough for upward air flow from said lower plenum chamber into said product container section, said product container section including a substantially cylindrical partition spaced above said air distribution plate/screen for dividing said product container section into an inner upbed area and an outer dowabed area, and an upwardly discharging spray nozzle mounted substantially centrally within said cylindrical partition, said method including the steps of positioning a cylindrical inner partition adjacent said distribution plate/screen and extending upwardly therefrom, surrounding said nozzle, and projecting upwardly to a level at least equal in height to said nozzle, and passing air upwardly through said air distribution plate/screen and through said cylindrical inner partition about said nozzle to shield the initial spray pattern developed by said nozzle against the entrance of particles moving upwardly through said upbed.

2. The method of reducing the processing time of a granulator/coater of the fluidized bed type including a product container section opening upwardly into an upper expansion chamber and downwardly into a lower plenum chamber through a generally horizontal air distribution plate/screen having openings formed therethrough for upward air flow from said lower plenum chamber into said product container section and wherein said product container section contains an upright cylindrical partition supported centrally therein spaced above said air distribution plate/screen and dividing said product container section into an inner upbed and an outer downbed, and an upwardly discharging spray nozzle mounted centrally with respect to said upbed in a lower portion thereof, said method including the steps of forming a radially confined and shielded column of air to flow upwardly about said nozzle from said air distribution plate/screen and to be

freely discharged into said upbed at an elevation generally vertically registered with the upper extremity of said nozzle.

3. The method of claim 1 wherein the shielding step comprises providing a barrier by positioning a partition about the spray nozzle, said partition having opposite open ends, one open end of said partition positioned away from the spray nozzle tip toward the direction of the discharging spray, the other open end of said partition positioned to surround a portion of the spray nozzle.

4. A method for shielding an initial spray pattern developed by a discharging spray nozzle situated within a fluidized bed processor, said fluidized bed processor including a container for containing particles to be processed, a fluidizing gas source, and a discharging spray nozzle having a spray nozzle tip within the container, the method comprising the steps of forming a fluidized bed within the container by passing fluidizing gas through the particles to be processed, discharging a spray from the discharging spray nozzle into the fluidized bed, and shielding the initial spray pattern developed by the discharging spray nozzle by providing a barrier surrounding the spray nozzle tip and oriented substantially parallel to the spray nozzle for preventing the particles to be processed from entering the initial spray pattern.

5. The method of claim 1, wherein the shielding step comprises providing a barrier formed from a column of gas about the discharging spray nozzle tip and directed substantially parallel with the spray nozzle, said column of gas extending beyond the spray nozzle tip in the direction of the discharging spray.

6. The method of claim 5, wherein the column of gas is formed from the fluidizing gas.

7. The method of claim 5, wherein the column of gas is substantially radially confined.

8. The method of claim 7, wherein the forming of the substantially radially confined column of gas comprises positioning a cylindrical partition having opposite open ends around the spray nozzle, passing gas through one end of the cylindrical partition and outward through the other end of the cylindrical partition, said other end positioned adjacent the spray nozzle tip, whereby the column of gas prevents the particles being processed from entering the initial spray pattern.

9. The method of claim 8, wherein the column of gas is formed from the fluidizing gas.

* * * * *

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Spray-coating of rhDNase on lactose: effect of system design, operational parameters and protein formulation

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Abstract

The feasibility of spray-coating fine lactose powders with recombinant human deoxyribonuclease (rhDNase) using a bench-top fluid-bed processor (STREA-1) was studied. The effect of operating parameters, system design and protein formulation on coating performance was evaluated and compared with a laboratory-scale, Würster processor (GPCG-1), as reported previously (Maa and Hsu, 1996a). Protein denaturation occurred during spray-coating in both processors, though to a lesser degree in STREA-1 than in GPCG-1. The cause of protein denaturation during coating was determined to be thermally induced and most likely occurred during drying. The combined effect of shear and heat on protein aggregation during atomization was found to be insignificant. GPCG-1 outperformed STREA-1 in terms of particle agglomeration and product yield. Particle agglomeration in the latter could be reduced by increasing the atomizing pressure and decreasing the liquid feed rate. Overall, this report demonstrates that it is feasible to use the bench-top fluid-bed processor for protein spray-coating, but the application on fine carriers ($< 100\ \mu m$) is limited.

Keywords: Aggregation; Agglomeration; rhDNase; Fluid-bed; Spray coating; STREA-1; Würster process

1. Introduction

Spray-coating is a useful alternative in producing pharmaceutical protein dosage forms for pulmonary, oral, or controlled delivery. A previous study has shown that it is feasible to spray-coat recombinant human deoxyribonuclease (rhDNase) onto a lactose microcarrier as small as 50 μm using a laboratory-scale spray-coater (Glatt GPCG-1) (Maa and Hsu, 1996a). Despite its robust mechanical integrity, spray-coated rhDNase suffered serious chemical denaturation (i.e. aggregation), which was attributed to thermal degradation. To closely examine the spray-coating process, we explored a smaller scale, bench-top spray-coater, STREA-1 (Niro, Columbia, MD).

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STREA-1 has been widely used in the pharmaceutical industry to perform fluid-bed drying, granulation and coating of solid low-molecular-weight drug powders (Olsen, 1989a,b). This coater came with two spraying designs, top-spray and bottom-spray. Both systems were evaluated in this study. The bottom-spray system was based on a Würster process, which has been known for spray-coating powders as small as 30–40 μm (Fukumori et al., 1988) using GPCG-1. As far as the production scale is concerned, STREA-1 can process 0.1–2 l of carriers, compared 0.5–5 l handled by GPCG-1.

Two major problems in protein spray-coating are particle agglomeration and protein denaturation. Thus, it is important to understand these issues and ultimately overcome them, in order to make protein spray-coating commercially feasible. Particle agglomeration is the most serious problem encountered in the spray-coating process (Fukumori et al., 1987, 1988, 1991a,b, 1992). Many factors play a role in particle agglomeration, including the nature and concentration of the coating material, as well as atomized liquid droplet size and size distribution (Fukumori et al., 1992). Fukumori et al. (1991a) found that the inertia of particle flow is crucial, particles larger than 100 μm possessing sufficient inertia to overcome the cohesive force amongst particles themselves and the adhesive force with the chamber wall. As such, fluidization performance can affect the particle flow pattern, thus affecting particle agglomeration.

Another important issue during spray-coating is product denaturation. It has been reported that rhDNase coating prepared by GPCG-1 suffered serious aggregation (20–30%), which was attributed to thermal degradation based on the hypothesis that the coating had been exposed to high temperatures (Maa and Hsu, 1996a). However, it was not shown whether this rhDNase denaturation occurred during atomization and/or during drying. Therefore, another objective of the present study was to further examine the cause of such aggregation and to understand how formulations affect product quality. Upon two-fluid atomization, the high-speed air generates shear stresses causing liquid dispersed into fine droplets with a large air–liquid interfacial area. The protein solution might also have encountered heat when passing through the nozzle head. Therefore, thermal stress, shear stress and air–liquid interface might all affect the protein during atomization. During drying, thin coating is first dried to a partially wet, viscous membrane (early-stage drying) and then to a dried solid layer (late-stage drying). In this study, experiments were performed to understand how atomization and drying affect rhDNase aggregation during spray-coating. The effect of protein formulation on coating performance was also discussed.

2. Materials and methods

2.1. Materials

2.1.1. rhDNase

Recombinant human deoxyribonuclease (rhDNase), a glycosylated protein with a molecular mass of 32.74 kDa, was produced at Genentech (South San Francisco, CA), using Chinese hamster ovary cell line. The concentrated rhDNase solution was prepared by ultrafiltration/diafiltration of an 11 mg/ml rhDNase bulk (containing 150 mM sodium chloride and 1 mM calcium chloride) using tangential flow filtration. This increased the rhDNase concentration to approximately 28 mg/ml and reduced salt concentration to approximately 10 mM. Lower rhDNase concentrations were prepared by dilution of the bulk with purified water. All solutions were filtered with a 0.22 μm filter prior to the experiments.

2.1.2. Lactose

The lactose powder (Pharmatose 100 M, DMV International, Fraser, New York) was sieved with US standard sieves (ASTM E-11) of 53, 125 and 250 μm on a sieve shaker (CSC Scientific) at 200 rpm for 1 h. Each load of sieving was 250–300 g. Only the fractions of 53–125 and 125–250 μm were used for the study. The yield of each fraction was approximately 40%.

2.1.3. Excipients

Calcium chloride ($CaCl_2$) was obtained from J.T. Baker in pellet form. Trehalose was obtained from Sigma. Polysorbate (Tween) 20 (Karshamns) was prepared into a 20% aqueous solution before being added to the protein solution. All materials were used as supplied.

2.1.4. STREA-1 spray-coater

STREA-1 (Fig. 1) is a bench-top laboratory fluid-bed dryer (Niro, Columbia, MD). It consists of a control unit, a drying chamber and a two-fluid nozzle. The control unit allows the operating time, the drying air flow, the air inlet temperature, the atomization pressure and the blow-out pressure to be controlled. The pressure drop across the filter (on the top of the spray chamber to retain particles) and across the air distributor (on the bottom of the spray column) has to be monitored in order to provide appropriate drying air flow to fluidize the carriers. The chamber associated with top-spray (Fig. 1b) is available in both polyacrylate (Plexiglas) and stainless steel, but only stainless steel in the bottom-spray design. Three vertical side-ports in the top-spray Plexiglas chamber are used for nozzle insertion. The bottom-spray chamber (Fig. 1a) comes with a vertical partition tube sitting on the top of a dish-shaped air distributor. The nozzle is located in the lower center of the partition tube. The atomization nozzle has an orifice diameter of 0.7 mm, where air and liquid are externally mixed.

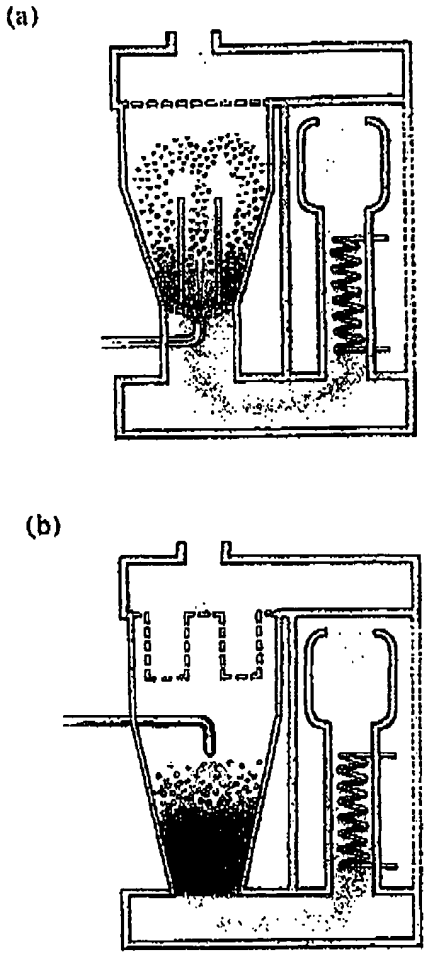

Fig. 1. The configuration of the bottom-spray (a) and the top-spray (b) STREA-1 systems.

2.1.5. GPCG-1 spray-coater

This system has been previously described (Maa and Hsu, 1996a). This device is a microprocess-controlled laboratory unit (Glatt Air Techniques, Ramsey, NJ). Generally, its loading capacity is 0.5–5 l depending upon the powder density. In the current study, atomization was performed using a 0.7 mm two-fluid nozzle and operated in a bottom-spray Würster mode.

2.2. Methods

2.2.1. Spray coating

Lactose powder was loaded into the fluidization chamber and fluidized with a drying air at a desired flow rate and temperature; then rhDNase solution was fed to the nozzle using a Masterflux pump (Console Drive, Cole Parmer). Fig. 1a shows a bottom-spray Würster system with a dished air-distributor plate and Fig. 1b shows a top-spray coater. When introduced upward through the plate, the high-velocity air lifts the powder in the chamber to a height depending on the air velocity, particle size and powder density.

In the bottom spray coater, the Würster chamber is partitioned by a draft tube which allows air to be moved faster inside than outside the tube. When particles enter the high-velocity spout, they are uniformly accelerated and physically separated from each other, facilitating uniform coating inside the tube. The process air that moves the particles also serves to dry the coating. When the airstream and particles clear the top of the partition, the air spreads out to fill the expansion chamber, and the particles settle out to the bottom of the bed outside the partition because of the lower air velocity. The settled particles then re-enter the partition to receive additional coating. The coating process continued like this for many cycles. The base operating conditions in this study were: 200 g of lactose powder and 400 ml of liquid, drying air flow rate (Q_D) of 40–50 m^3/h, atomizing air pressure of 17.4 psi, liquid feed rate (Q_L) of 6.7 ml/min, and drying air inlet temperature (T_{inlet}) of 90°C.

2.2.2. Spraying and spray-drying

The top-spray STREA-1 system was used without fluidization of microcarriers for the spray-drying and the spraying study. The spraying study was to investigate the combined effect of shear stress and air–water interface on the protein. Spray-drying was to examine how thermal stress combined with shear stress and an air–liquid interface affects the protein. In both cases, protein solution was sprayed into a Plexiglas chamber. For spraying, the atomized droplets were collected in a beaker sitting under the chamber. For spray-drying, the sprayed droplets were dried on the wall of the chamber and were then redissolved into water for analysis. To examine the thermal effect, similar spraying experiments were conducted using protein solutions preheated to 60°C in a jacked glass vessel circulated with 50/50 water/ethylene glycol.

A mass flow meter (Model 820, Sierra Instrument) was installed right before the nozzle to measure the volumetric flow rate of the atomizing air. The air flow speed was calculated based on the air flow rate and the opening area between the nozzle orifice and the nozzle cap. The outside diameter of the orifice and the diameter of the opening in the nozzle cap were measured to be 2.0 and 2.7 mm, respectively. Accordingly, the annular area of this nozzle-tip opening was calculated to be 0.022 cm^2.

2.2.3. Shear experiment by rotor/stator homogenizer

A Virtishear homogenizer (Virtis, Templest IQ) consisting of a digital display microprocessor control, an overhead drive and a homogenizing shaft (1 cm diameter) was used. The shaft tip is a rotor/stator assembly. The configuration of the assembly has been detailed previously (Maa and Hsu, 1996d). When the rotor rotates, the assembly draws the liquid in from the bottom of the shaft and sends it out through four 0.4 cm openings equally spaced above the rectangular slots for circulation. The rotational speed can be steadily controlled between a range of 5000 and 25 000 rpm. The emulsification vessel is a jacketed glass container with an inner cylinder 6 cm high and 2.5 cm in diameter. The inner tube holds approximately 50 ml of aqueous solution.

2.2.4. Incubation experiments

Thermal stress on the protein was investigated by incubating 20 ml of protein solution in a jacketed glass vessel circulated with 50/50 water/ethylene glycol at a pre-set temperature. Protein aggregation was analyzed after each incubation period.

2.2.5. Protein and powder characterizations

2.2.5.1. Size exclusion chromatography-high pressure liquid chromatography (SEC-HPLC). Samples of rhDNase were diluted to 1 mg/ml using water and 100 μl was injected into a silica-based Tosoh TSK 2000SW XL column (7.8 mm internal diameter × 30 cm length; particle size 5 μm). The mobile phase was a mixture of 5 mM HEPES, 150 mM NaCl and 1 mM $CaCl_2$ at pH 7.0, and was pumped at a flow rate of 1 ml/min. The run time was 15 min. Protein concentration was measured by optical absorption at 280 nm.

2.2.5.2. UV spectrophotometry. The amount of insoluble rhDNase aggregates was determined by measuring the difference in protein concentration (Kontron Uvikon 860) of the reconstituted sample

before and after 0.22 μm filtration (Millipore, GSVP) at a wavelength of 280 nm. Insoluble aggregates less than 0.22 μm in size were not necessarily removed by filtration. All measurements were made referenced against pure water.

2.2.5.3. Product yield and rhDNase loading determination. The yield of the process was determined based on the ratio of the amount of the powder recovered after spray-coating to that initially loaded by weight. The amount of rhDNase loaded on lactose was determined by analyzing protein concentration in each reconstituted solution.

2.2.5.4. Particle agglomeration. Each pre-weighed powder sample was sieved in an autosieve (GilSonic, Gilson Company) at 60 rpm for 10 min (ramping both up and down in 1 min). Particles of sizes ranging from 53 to 125 μm were sieved into three size groups: greater than 125 μm, 45–125 μm, and smaller than 45 μm. Particles of sizes ranging from 125 to 250 μm were also sieved into three size groups: greater than 250 μm, 125–250 μm and smaller than 125 μm. The fraction of the powder in each size range was determined by weight.

2.2.5.5. Scanning electron microscopy (SEM). The surface morphology of coated powder was examined using a Philip SEM system (Model 525M). Powder samples were mounted to a sample stub, and coated under a high vacuum (< 0.05 mtorr) with a layer of 10 nm gold–platinum. All samples were scanned at a voltage of 4.0 kV.

3. Results and discussion

Spray coating is a complex process consisting of three major operations: fluidization, atomization and drying. Table 1 summarizes all the parameters involved in the spray-coating process. These parameters are either operating variables, system design variables, or properties associated with the powder or the coating liquid. In all steps, the volumetric flow rates of drying air (Q_D), atomizing air (Q_A), and the liquid feed (Q_L), in addition to the drying air inlet temperature (T_{inlet}), are the most basic operating variables. Spray design includes the top-spray and the bottom-spray systems. The drying chamber associated with top-spraying was made of either polyacrylate or stainless steel. Only the stainless steel chamber was available for the bottom-spraying system. Powders used in this study were lactose with two size fractions: 53–125 and 125–250 μm. The liquid feeds used were pure water, aqueous lactose solution and aqueous rhDNase. Surface tension, viscosity and density of the aqueous lactose and rhDNase solutions were determined to be not significantly different from those of pure water (data not shown).

Coater performance was evaluated based on three criteria: product quality, product yield and production time. An optimum operation will produce a quality product with minimal particle agglomeration and protein denaturation at a high yield in a short production time. GPCG-1 met

Table 1
Parameters involved in regular spray-coating

Fluidization	Drying air flow rate[a] Spray design[b] (top-spray vs. bottom-spray) Chamber material[b] (stainless steel, glass, polymer, etc.) Powder size and density[d] (lactose, 53–125 and 125–250 μm) Particle surface and charge[d] Coating material[b] (rhDNase, lactose)
Atomization	Atomizing air flow rate[a] Liquid feed rate[a] Nozzle design[b] (external vs. internal mixing) Liquid viscosity[c] Liquid surface tension[c] Liquid density[c]
Drying	Drying air inlet temperature[a] Drying air flow rate[a] Liquid feed rate[a] Atomizing air flow rate[a] Liquid concentration[c]

[a] Operating variables.
[b] System design.
[c] Liquid properties.
[d] Particle properties.

these criteria, except that the protein in the coating was highly aggregated (Maa and Hsu, 1996a). Therefore, the coating performance of STREA-1 was evaluated based upon available design options using operating variables comparable to those used in GPCG-1 operation.

3.1. Fluidization performance

The lactose powder of 125–250 μm in size was fluidized using a top-spray polyacrylate chamber (Plexiglas) at the base conditions. The drawback of this system is that lactose particles tended to stick to the wall of the chamber due to electrostatic force, making fluidization impossible. When replaced by a stainless steel chamber, the problem of powder sticking to the wall was much alleviated. A voltmeter (Fluke, 8060 A) was used to measure the voltage difference (ΔV) between the chamber and the electrically-ground metal stand below the chamber. The value of ΔV was determined to be 64 mV for the polyacrylate chamber versus 4 mV for the stainless steel chamber, consistent with the finding above. Like the electrically-ground chamber ($\Delta V = 0$), the stainless steel chamber (4 mV) did not affect the fluidization pattern because steel conducts excessive static charge to the air. Another approach to reducing the electrostatic force was to incorporate an air ionization cartridge (Model 6110a, Ion Systems) before the nozzle. This air ionizer was designed to neutralize static charge on surfaces with clean, dry compressed air sources. The effect of air ionization on particle agglomeration after coating will be discussed later.

Another problem with fluidization is the control on drying air and atomizing air flow. Increased air flow resulted in increased air filter resistance (due to fouling by small particles), which in turn limited the overall air flow and fluidization performance. The maximum filter resistance measured was approximately 300 mm water (0.43 psi). This limit was often exceeded when lactose powders of less than 250 μm were fluidized. Because of the bag filter fouling problem, STREA-1 is less suitable for spray-coating of fine powders than GPCG-1.

3.2. Coating performance between GPCG-1 and STREA-1

Table 2 summarizes the conditions and the results for spray-coating of lactose powder (125–250 μm) using GPCG-1 and STREA-1. The conditions between the two preparations were matched. STREA-1 resulted in less rhDNase aggregation than GPCG-1, especially in the formation of insoluble aggregates. The cause for protein denaturation was previously attributed to the thermal effect (Maa and Hsu, 1996a). The more detrimental effect by GPCG-1 is probably because each coating/drying cycle is longer as a result of a longer path of particle flow in a larger fluidization chamber.

Particle agglomeration was a serious concern for STREA-1. Fig. 2a is the SEM indicating that, prior to spray-coating, lactose particles were discrete. Upon coating using the conditions listed in Table 2, some of these particles became agglomerated (Fig. 2b). Agglomeration occurred due to coating adhesion between particles, overcoming the dispersion force by the inertia of particle movement in the chamber. Table 3 summarizes the production yield and the wt.% of agglomerated lactose coated using GPCG-1 and STREA-1. Of four batches prepared by GPCG-1, coarse lactose (125–250 μm) had lower agglomeration

Table 2
Comparison of spray-coating using GPCG-1 and STREA-1

	GPCG-1	STREA-1
Conditions		
Batch size (kg)	0.5	0.2
DNase conc. (mg/ml)	28	20
Lactose powder (μm)	125–250	125–250
Inlet temp. (°C)	75	75
Outlet temp. (°C)	35	40
Drying air volume (m^3/h)	46	45
Feed (g/min)	10	6.8
Atomizing pressure (psi)	43.5	43
Coating time (min)	97	90
Results		
Theoretical loading (%)	5	6
Actual loading (%)	5.58	5.52
Soluble aggregate (%)	10.6	4.2
Insoluble aggregate (%)	24.4	0

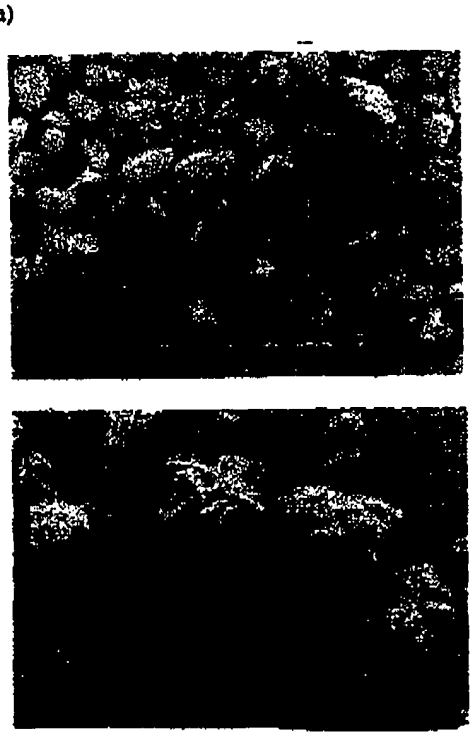

Fig. 2. SEM for lactose powder (125–250 μm) before spray-coating (a) and after spray-coating (b) using the condition for STREA-1 listed in Table 2.

and higher yield than fine lactose (53–125 μm). In general, GPCG-1 produced powders of lower agglomeration and higher yield than STREA-1. Coating materials affected agglomeration significantly. Powder coated with pure rhDNase is less sticky than that coated with lactose-containing rhDNase or lactose itself. The addition of either Tween 20 or $CaCl_2$ made rhDNase-coated powder stickier. In view of the fact that pure water (without any coating material) still caused agglomeration, it suggests that the inertia of particle movement played a role. The larger the fluidization chamber, the greater the inertia of particle flow. Therefore, larger coaters produce less agglomerated particles.

Table 4 suggests that system design (top-spray versus bottom-spray) affects agglomeration and production yield significantly. Top-spraying resulted in higher agglomeration and lower production yield than bottom-spraying. The bottom-spray coating (Fig. 1a) was based on a Würster process where the liquid was atomized into a partition tube. When pure water was sprayed, the yield by top-spraying was significantly lower (27% versus 87% in bottom-spraying), because of particle attachment to the wall of the chamber. Particle build-up on the chamber wall was significant for top-spraying regardless of coating conditions, suggesting that this design is not suitable for microcarriers of less than 250 μm. Agglomeration was found to be higher in the fine powder (53–125 μm) than in the coarse one (125–250 μm). It is because large particles possess higher inertia and have a better chance to overcome the adhesive force during coating than small particles, suggesting it is difficult to coat fine powders using small coaters. The use of an air ionization cartridge could not alleviate agglomeration, suggesting that particle agglomeration was not dominated by the electrostatic force between lactose particles.

The approach to reducing agglomeration was to increase the atomization pressure (atomizing air speed) and to decrease the liquid feed rate (Table 4). Increasing the atomizing air speed not only decreased the droplet size of the spray but also increased the inertia of particles flowing through the partition tube. Fukumori et al. (1992) concluded that even a small amount of coarse droplets could lead to significant agglomeration. Upon spraying the rhDNase/lactose solution using a high atomizing pressure (17.4 psi) and a low liquid feed rate (2.5 ml/min), the small lactose powder agglomerated (35.8%) more significantly than the large powder (10.4%), suggesting that the relative size between sprayed droplets and fluidized particles was important. The higher the particle/droplet ratio, the lower the agglomeration. However, using a high air pressure resulted in more particle deposition on the filter, this in turn increased the filter resistance and limited the fluidization performance. Although resulting in a decrease in agglomeration, the low feed rate increased the processing time and made the process become impractical.

Table 3
Spray-coating[a] of various aqueous solutions on lactose using GPCG-1[b] and STREA-1

Coater	Lactose (μm)	Coating solution (mg/ml)	Agglomeration (%)	Yield (%)
GPCG (bottom)	53–125	DNase (28)	7.6 (>125 μm)	92
GPCG (bottom)	125–250	DNase (28)	1.5 (>250 μm)	100
GPCG (bottom)	53–125	DNase (14)	2.8 (>125 μm)	86
GPCG (bottom)	125–250	DNase (14)	0.3 (>250 μm)	100
STREA (bottom)	125–250	Pure water	13.3 (>250 μm)	89
STREA (bottom)	125–250	Lactose (20)	85.4 (>250 μm)	91
STREA (bottom)	125–250	rhDNase (20)	35.7 (>250 μm)	86
STREA (bottom)	125–250	DNase (7.5)/lactose (3.2)	87.6 (>250 μm)	87
STREA (bottom)	125–250	DNase (8.8)/Tween (1)	99.1 (>250 μm)	93
STREA (bottom)	125–250	DNase (20)/$CaCl_2$ (20 mM)	94.8 (>250 μm)	85

[a] Spray-coating condition: Q_L = 6.8 ml/min, atomizing pressure = 8.7 psi, Q_D = 40–50 m^3/h, T_{in} = 90°C, 200 g of lactose powder and 400 ml of solution.
[b] Data from Maa and Hsu (1996a).

3.3. Causes for rhDNase denaturation

Spray-coating and spray-drying share the same atomization principle. Both use a two-fluid nozzle to atomize liquid into fine droplets due to high shear stress generated by high-speed atomizing air. These fine droplets possess a large air–liquid interfacial area and are exposed to a high temperature upon contacting the hot air. In addition, protein solution might have been subjected to thermal stress when flowing through the hot nozzle head. Therefore, heat, shear stress and air–liquid interface may all affect the protein during atomization. Although spray-coating and spray-drying share a similar drying principle, their thermal stress might be different. During the early stage of these operations, the coating (as the result of the spray-coating process) or the droplet (as the result of the spray-drying process) is first dried to a partially wet, viscous liquid and is further dried to a solid film or particle, respectively, during late-stage drying. Upon coating, the drying film continues to be exposed to the hot air prior to the next coating cycle (referred to as dry heating herein). In addition, the wet coating and droplet have experienced a longer exposure to a higher average temperature than the droplet during spray-drying (Maa and Hsu, 1996a). Therefore, drying and atomization might be the two major causes for protein denaturation and will be discussed in the following.

3.4. Effect of dry heating

To examine the thermal effect on a dried coating, i.e. the dry heating stage, rhDNase-coated (12% loading) lactose powder (125–250 μm) prepared by GPCG-1 (Maa and Hsu, 1996a) was further fluidized and dried in STREA-1 for 1 h using a drying air of 90°C at a flow rate of 45 m^3/h. No additional aggregation was found after this extended dry heating, suggesting that the protein is stable as long as it is dry.

3.5. Thermal denaturation of rhDNase

It was previously determined using scanning microcalorimetry (Maa and Hsu, 1996a) that rhDNase (in the liquid state) thermally denatured with a peak onset temperature at 52°C and a maximum peak temperature at 63°C. Also, $CaCl_2$ was found to thermally stabilize rhDNase, upshifting both temperatures by approximately 10°C. The effect of high temperatures on rhDNase aggregation is shown in Fig. 3a, where the protein solutions (20 mg/ml) with and without Ca^{+2} were incubated for 10 min in a preheated jacketed glass vessel. When the incubation temperature was lower than the peak onset temperature, e.g. 50°C, rhDNase remained intact. Above the onset temperature, the degree of rhDNase aggregation increased with increasing temperatures and reached almost 100% denaturation at

Table 4
Comparison of top-spray and bottom-spray configurations on spray-coating of various aqueous solutions on lactose[a]

STREA-1	Lactose (μm)	Coating solution (mg/ml)	Agglomeration (%)	Yield (%)
Top	125–250	Pure water	11.0 (>250 μm)	27
Top[b]	125–250	Pure water	33.9 (>250 μm)	50
Bottom	125–250	Pure water	13.3 (>250 μm)	87
Bottom[b]	125–250	Pure water	0.0 (>250 μm)	80
Bottom[c]	125–250	Pure water	0.1 (>250 μm)	83
Bottom	125–250	DNase (7.5)/lactose (3.2)	87.6 (>250 μm)	87
Bottom[b]	125–250	DNase (7.5)/lactose (3.2)	10.4 (>250 μm)	83
Bottom[d]	125–250	Pure water	60.8 (>250 μm)	95
Bottom	53–125	Pure water	60.5 (>125 μm)	88
Bottom[b]	53–125	Pure water	0.5 (>125 μm)	74
Bottom[b]	53–125	DNase (7.5)/lactose (3.2)	35.8 (>125 μm)	85

[a] Unless otherwise stated, the spray-coating conditions are: $Q_L = 6.8$ ml/min, atomizing pressure = 8.7 psi, $Q_D = 40–50$ m³/h, $T_{in} = 90$°C, 200 g of lactose powder and 400 ml of solution.
[b] $Q_L = 2.5$ ml/min and atomizing pressure = 17.4 psi.
[c] $Q_L = 6.8$ ml/min and atomizing pressure = 17.4 psi.
[d] Air ionization cartridge in use.

70°C. In the case of protein containing Ca^{2+} (20 mM), rhDNase remained stable at 65°C. The effect of incubation time on aggregation of rhDNase containing no Ca^{2+} at 60 and 65°C is also shown in Fig. 3b, indicating that protein denaturation increased with longer exposure to high temperatures.

3.6. Atomization effect

The effect of atomization on rhDNase denaturation was evaluated based on the combined influence of shear (defined as the product of shear rate and the time subjected to this shear rate), air–liquid interface, and heat. It was previously determined that the shear effect alone (Maa and Hsu, 1996b) and the combined effect of shear and air–liquid interface (Maa and Hsu, 1996c) on rhDNase aggregation was not significant. Those two studies were modeled based on a rotor/stator homogenization system. In spray-coating, the protein might encounter heat during atomization (flowing through a hot nozzle head). Therefore, the effect of elevated temperature, in combination with shear and an air–liquid interface, was evaluated using the rotor/stator homogenization model to simulate the atomization process.

Fig. 4 shows rhDNase aggregation as a function of shear using 20 ml of rhDNase solution homogenized at 24 000 rpm at 50 and 60°C in the presence of air–liquid interface and at 60°C under the air-free condition. The rhDNase solution containing 20 mM $CaCl_2$ was homogenized at 60°C in the presence of air. To determine the extent of denaturation contributed by shear alone, all the data collected at 60°C during homogenization was adjusted by subtracting the aggregation contributed by thermal incubation at 60°C (data from Fig. 3). Highly-sheared rhDNase remained intact at 50°C. However, sheared rhDNase became susceptible to aggregation at 60°C with or without air–liquid interface, suggesting that shear stress facilitated aggregation when the rhDNase was thermally unstable and that the air–liquid interface alone played only a minor role. The data show that above the onset peak temperature, rhDNase was susceptible to aggregation unless $CaCl_2$ was present. This result confirms that, when rhDNase was thermally stable, the effect of shear stress and air–liquid interface on rhDNase integrity was not significant.

To consider the possible influence of shear stress, air–liquid interface and heat on rhDNase aggregation during atomization, rhDNase solu-

tion was preheated to 60°C and was atomized at different atomizing pressures into a Plexiglas chamber. During two-fluid atomization, high shear rates were induced by the relative velocity between the liquid feed and the atomizing air (Masters, 1991). The results for the atomizing pressure, the volumetric flow rate of the atomizing air and the air flow speed were determined experimentally and are listed in Table 5. The mean Sauter diameter of the droplet, defined by $\Sigma n_i d_i^3 / \Sigma n_i d_i^2$, was calculated (listed in Table 5) according to the equation (Katta and Gauvin, 1975):

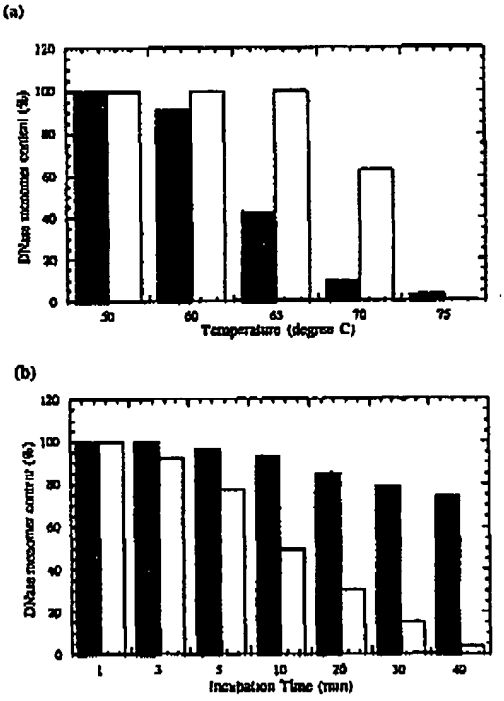

Fig. 3. Aggregation of rhDNase as a function of incubation temperature (a) and time (b). In (a), rhDNase solution (1 ml at 20 mg/ml) (shaded bar) and the same solution containing 20 mM of $CaCl_2$ (empty bar) were incubated in a Lauda circulator at different temperatures for 10 min. In (b), rhDNase solutions (20 ml) were incubated in a jacketed glass vessel at 60°C (shaded bar) and 65°C (empty bar) as a function of incubation time.

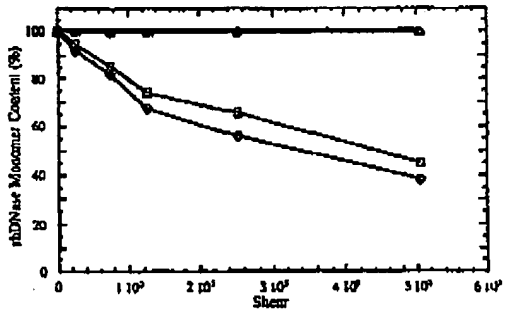

Fig. 4. The combined effect of shear, air–liquid interface and heat on rhDNase aggregation. The rhDNase solution (20 ml at 8 mg/ml) was preheated to 50°C (○) and 60°C (◇) for 10 min in a jacketed glass vessel for homogenization at 24000 rpm. The same solution heated to 60°C was homogenized without the air–liquid interface (□). Protein solution containing 20 mM of $CaCl_2$ was preheated to 60°C, and then homogenized under the same condition (△).

$$d_{v_s} = \frac{585}{V_{rel}}\left(\frac{\sigma}{\rho}\right)^{0.5} + 597\left(\frac{\mu}{\sqrt{\sigma\rho}}\right)^{0.45}\left(\frac{1000Q_L}{Q_a}\right)^{1.5} \quad (1)$$

where the drying air flow rate ($Q_a = 40$ m^3/h), the liquid flow rate ($Q_L = 6 \times 10^{-4}$ m^3/h), liquid viscosity ($\mu = 0.01$ P), surface tension ($\sigma = 68$ dyne/cm) and liquid density ($\rho = 1000$ kg/m^3) were used for rhDNase solution (Maa and Hsu, 1996c). Based on the data in Table 5, the relationship between V_{rel} and atomizing pressure is linear, and the relationship between V_{rel} and d_{v_s} can be expressed as $d_{v_s} = 412 V_{rel}^{-1.13}$ for the aqueous rhDNase system. The power term of V_{rel} is consistent with the coefficient of -1.14 reported by Masters (1991). This relationship is also similar to that established for the rotor/stator homogenization system (Maa and Hsu, 1996d): $d_p \propto V_{homo}^{-1.14}$, where d_p is the size of liquid droplets in a liquid–liquid emulsion and V_{homo} the homogenization intensity. This suggests that the velocity in both systems, V_{homo} and V_{rel}, has a similar effect on the droplet size. Therefore, it is valid to select this homogenization system as a hydrodynamic model to estimate the shear and the shear rate associated with atomization. In this homogenization system, the average shear rate ($\langle\gamma\rangle_{homo}$) is linearly proportional to V_{homo} (Maa and Hsu, 1996b), i.e. $d_p \propto \langle\gamma\rangle_{homo}^{-1.14}$. By analogy, i.e. assuming

Table 5
The relationship between atomizing pressure and experimentally determined air flow rate and air flow speed[a]

Atomizing pressure (psi)	Volumetric flow rate (Q_a) (m^3/h)	Air speed (V_{rel}) (m/s)	Droplet size (d_{vs})[b] (μm)
5.8	0.52	66.2	3.88
11.6	0.71	92.0	2.49
17.6	0.93	109.3	1.88
23.2	1.15	147.5	1.51
29.0	1.26	174.2	1.29

[a] Atomization was achieved using an external-mixing nozzle (0.7 mm orifice).
[b] Mean Sauter diameter, $\Sigma n_i d_i^3 / \Sigma n_i d_i^2$, calculated using Eq. (1) (Katta and Gauvin, 1975).

$V_{rel} \propto \langle \gamma \rangle_{atom}^{-1.13}$, $\langle \gamma \rangle_{atom}$ should be comparable to $\langle \gamma \rangle_{homo}$ to obtain a similar size of droplets. Therefore, the shear rate induced during atomization might be equivalent to that induced by rotor/stator homogenization (in the range 10^4–$10^5\ s^{-1}$). However, the duration of atomization shear was very short (< 0.1 s) (Masters, 1991) compared with the duration in the rotor/stator homogenization experiment .

Based on the information above, the effect of atomization and homogenization on rhDNase aggregation was compared. For atomization, rhDNase solution (8 mg/ml) was preheated to 60°C for 10 min and then was sprayed into a Plexiglas chamber at three different pressures, 8.7, 14.5 and 29 psi. The results (Fig. 5) are compared with the aggregation profile for the protein homogenized at 60°C (Fig. 3). At 52 psi (atomizing air velocity = 312.4 m/s), it resulted in 4% rhDNase aggregation, corresponding to a shear of approximately 1×10^4 on the homogenization curve. This confirms our estimate that the shear experienced by the protein upon atomization is low, even though the shear rate might be high. It also suggests that denaturation due to the combined effect of shear, air- liquid interface and heat during atomization (either in GPCG-1 or STREA-1) was not significant, particularly at low atomizing air flow rates.

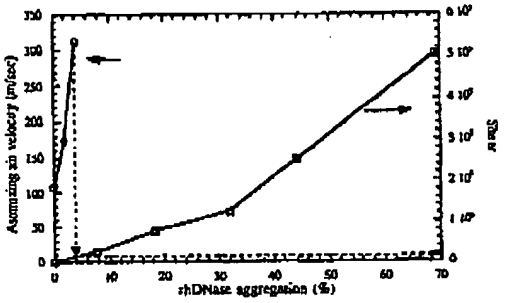

Fig. 5. Aggregation of rhDNase induced by shear during rotor/stator homogenization (□) and induced by the relative air–liquid velocity during atomization (○).

3.7. Formulation effect on thermal denaturation

To get a clearer picture of rhDNase degradation during spray-coating, we conducted a series of experiments to isolate the event potentially responsible. Results of this set of studies are shown in Table 6. In Expt. # 1, protein solution was atomized into ambient temperature. It confirms that stress associated with shear and the air–liquid interface had no effect on the protein. In Expt. # 2, protein solution was preheated to 60°C prior to atomization; the result suggests that the combined stress due to heat, shear and air–liquid interface during atomization on protein aggregation was not significant. Expt. # 3 imitated the spray-drying of a preheated rhDNase solution (60°C) in drying air initially at 90°C. This resulted in 2% aggregation, suggesting that although the protein solution might had been exposed to a temperature beyond its onset point of the denaturation peak during spray-drying, the actual damage to the protein may be less significant compared with spray-coating because of a relatively short period of exposure. In expt. # 4, rhDNase solution (no preheating) was spray-coated onto a lactose powder and produced approximately 6% of aggregates, suggesting that denaturation occurs mostly during drying. As hy-

Table 6
Five experiments for understanding the thermal effect during spray-coating using a STREA-1 processor

Experiment	1	2	3	4	5
DNase conc. (mg/ml)	10	10	10	10	10
Preheating (60°C) for 10 min	No	Yes	Yes	No	No
$CaCl_2$ (mM)	0	0	0	0	20
Atomization	Yes	Yes	Yes	Yes	Yes
Drying	No	No	Yes	Yes	Yes
Inlet temp. (°C)	—	—	90	90	90
Drying air volume (m^3/h)	—	—	40	40	40
Lactose (kg)	No	No	No	0.2	0.2
Soluble aggregate (%)	0	0[a]	2[a]	6	<1

All solutions were atomized at 17.4 psi and delivered at 5 ml/min.
[a] After subtraction of the aggregation due to preheating.

pothesized earlier (Maa and Hsu, 1996a), the inner side of the coating experiences a higher temperature than the outer side of the coating. Therefore, when the coating is partially wet (early-stage drying), protein in the inner side is prone to degradation. Expt. # 5 is the repeat of expt. # 4 except for the protein solution, which contained 20 mM of $CaCl_2$. In this experiment, aggregation was significantly reduced, confirming that rhDNase aggregation is thermally induced and can be prevented by using appropriate excipients to stabilize the protein.

Since the performance of STREA-1 was limited due to lack of sufficient inertia force, the formulation effect on coating quality (Table 7) was demonstrated using a GPCG-1 coater which had shown a good coating performance in particle agglomeration and product yield but exerted significant thermal stress. Formulations of rhDNase containing Ca^{+2}, trehalose, or Tween 20 were used. The latter two represent the sugar and the surfactant, which are commonly used to stabilize proteins during long-term storage. As shown earlier, Ca^{+2} reduced rhDNase aggregation (both soluble and insoluble) significantly. Addition of trehalose and Tween 20 could help reduce insoluble aggregation as well. However, the addition of these excipients caused serious particle agglomeration. When trehalose concentration was increased from 14 to 33 mg/ml, particle agglomeration increased from 7.1 to 33.5%. Agglomeration significantly increased to 80% when a small amount of Tween 20 was added (0.2 mg/ml final concentration). It suggests that the selection of formulations is crucial to the protein spray-coating process.

4. Conclusions

It is feasible to spray-coat rhDNase onto a lactose powder using the Würster (bottom-spray) STREA-1 processor because of its unique particle flow pattern. In general, STREA-1 is a more ideal coater for large carriers than small carriers and it can serve as a good model for understanding the protein spray-coating process. STREA-1 yields a coating of improved protein quality, but particle agglomeration is significant compared with the GPCG-1 processor. The degree of particle agglomeration strongly depends on coating materials and operating parameters. The most important operating parameter in the STREA-1 system are the atomizing pressure and the liquid feed rate. Aggregation of rhDNase during coating is thermally induced, mostly during the early-stage drying rather than during atomization, because shear involved in atomization is low. The effect of shear on rhDNase aggregation becomes significant only when rhDNase is heated to beyond its onset point of the thermal denaturation peak. This study also demonstrates that the selection of spray-coating formulation (e.g., sugar and surfactant) is essential to the balance between protein quality and physical properties of the powdered product.

Table 7
Formulation effect on rhDNase aggregation and particle agglomeration during spray-coating of lactose powder (53–125 μm) using a GPCG-1 coater[a]

Formulation	A	B	C	D	E
rhDNase conc. (mg/ml)	14	14	14	14	14
Excipients	No	$CaCl_2$	$CaCl_2$/trehalose	$CaCl_2$/trehalose	$CaCl_2$/trehalose/Tween 20
Conc. (mg/ml)	0	1	1/14	1/33	1/33/0.2
Loading (%)	5	2.5	2.5	2.1	2.1
Agglomeration (%)	2.8	4.5	7.1	33.5	79.2
Soluble aggr. (%)	17.3	2.2	2.5	5.6	5.4
Insoluble aggr. (%)	26.5	5.5	2.0	2.0	<1

[a] Spray-coating conditions: T_{in} at 75°C, Q_L at 10 ml/min, Q_D at 40 m³/h and Q_A at 1.1 m³/h.

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